Supplementary information

Sequence-specific inhibition of microRNA via CRISPR/CRISPRi system

Yicheng Zhao, Zhen Dai, Yang Liang, Ming Yin, Kuiying Ma, Mei He, Hongsheng Ouyang, Chun-Bo Teng

Supplementary Figure S1

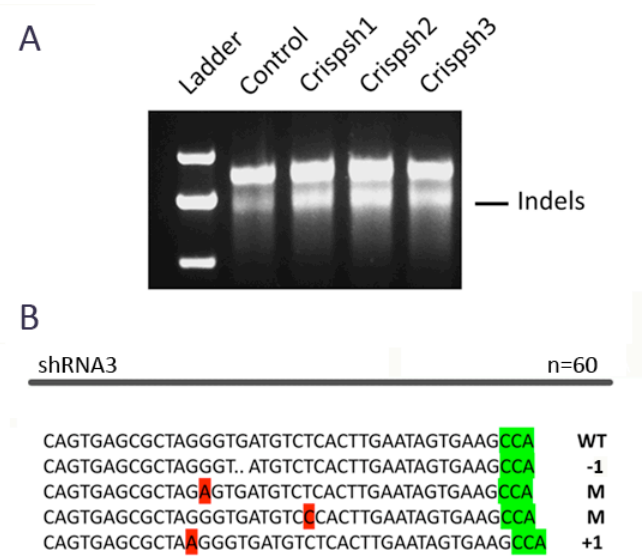

Figure S1. Cas9/gRNA-mediated DSBs at the target site in NIH3T3.

(A). Cleavage assays were performed 24 h post-infection. Indels were detected in reporter cells treated with Crispsh1, 2 or 3 by comparison with control samples, which show a weaker signal for the smaller band.

(B). Sequencing results of the targeted fragments from 60 T-A colonies amplified from a reporter 3 sample in which three mutations were detected. Mutations are highlighted in red, and PAM is highlighted in green.

Supplementary Figure S2

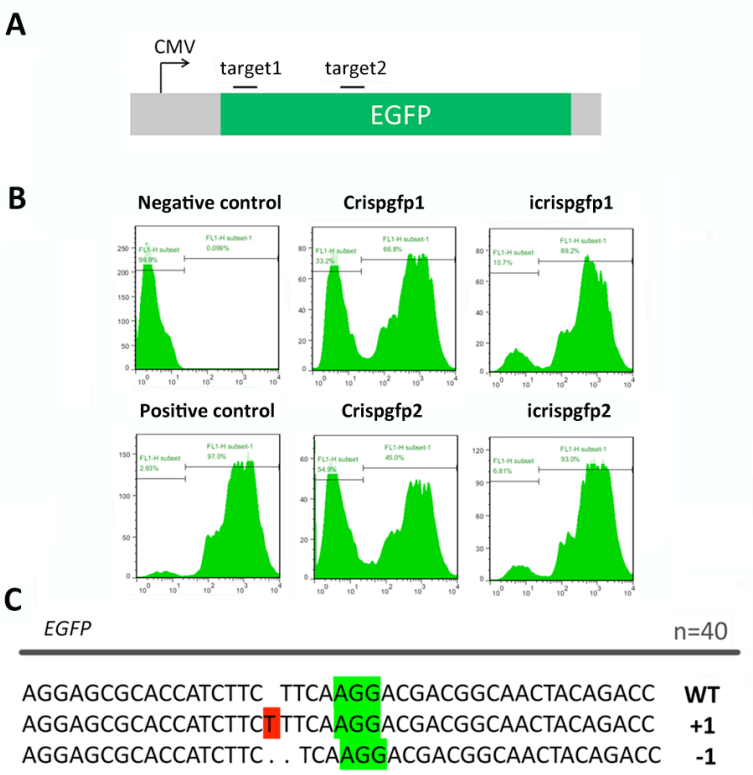

Figure S2. CRISPR and CRISPRi targeting of *EGFP* in PK-15.

(A). Schematic representation of two crRNAs binding to *EGFP* CDS. Four vectors were prepared including Crispgfp1-2 and iCrispgfp1-2.

(B). Representative flow cytometry histograms of EGFP-positive and EGFP-negative cells were chosen as controls. The fluorescence intensity distribution in Cas9/dCas9-treated clonal cells indicated inhibitory effects by CRISPR and CRISPRi.

(C). We obtained 40 T-A colonies of targeted fragments amplified from the Crispgfp2-treated EGFP-positive clonal PK-15 cells. Sequencing indicated 2 mutations 5 nt from the PAM site (shown in green).

Supplementary Figure S3

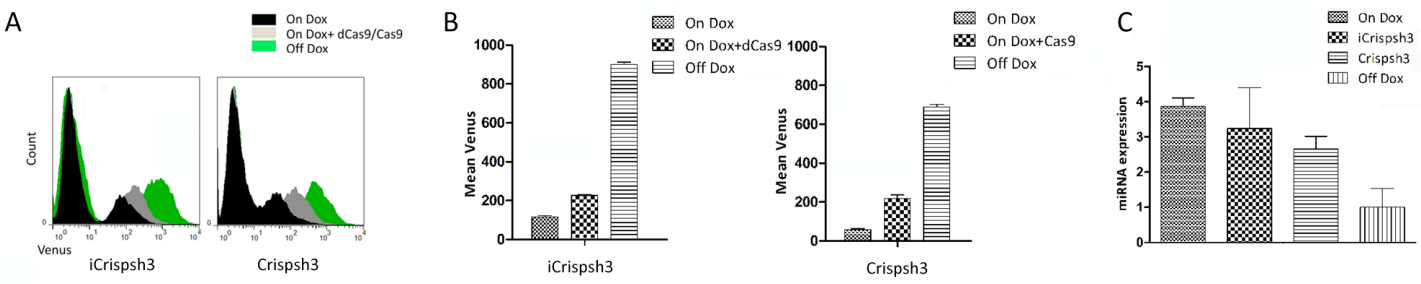

Figure S3. Inhibition of shRNA3 in PK-15 cells using CRISPR and CRISPRi.

(A). PK-15 cells were transfected with iCrispsh3/Crispsh3 and with or without Dox treatment (On/Off Dox). The fluorescence intensity distribution after 60 h indicated repression of shRNA3 by CRISPRi and CRISPR. The leftmost peaks represent uninfected cells.

(B). Relative analysis histograms were generated from FCM. The shRNA3 inhibition levels were 20% and 25% with CRISPRi and CRISPR, respectively. The values shown are the means of three replicates.

(C). Histograms calculated from the qPCR data indicated a repressive effect on miRNAs (20-30% fold) due to CRISPRi and CRISPR. The values shown are the means of three replicates.

Supplementary Table S1.

Name of Gene	DNA sequences of crRNA (5'-3')
shRNA1(PTEN.1523)	GGTGAAGATATATACATCTG
shRNA2(PTEN.2049)	ATTCACAAATTATACATCTG
shRNA3(BRAF.5053)	GTCTCACTTGAATACATCTG
EGFP	1, GACCAGGATGGGCACCACCC 2, GGAGCGCACCATCTTCTTCA
miR-21	ATGTTGACTGTTGAATCTCA
miR-30a-5p	GAAGCTGTGAAGCCACAAAT
miR-17-92	TGGTCACAGCTTCAGTCCCA

Supplementary Table S2.

Name of Gene	Primers (5'-3')
U6 (in common)	CTCGCTTCGGCAGCACA AACGCTTCACGAATTTGCGT
shRNA1(PTEN.1523)	CGGTATATCTTCACCTTTAGCTGGC
shRNA2(PTEN.2049)	GCCGTAATTTGTGAATGCTGATCTTC
shRNA3(BRAF.5053)	CGTTCAAGCGAGACATCACCCTAT
EGFP	GTTTAGTGAACCGTCAGATCCG GACTTGTACAGCTCGTCCATG
miR-21	GCTAGCTTATCAGACTGATGTTGA
miR-30a-5p	TGTAAACATCCTCGACTGGAAGA
miR-19a	GCTAGTTTTGCATAGTTGCACTAC
miR-20a	GTAAAGTGCTTATAGTGCAGGTAG
miR-92-1	AGGTTGGGATTTGTCGCAATGCT